

Claims:

1. A diagnostic method for the detection of the 5T, 7T and 9T alleles in intron 8 of the human CFTR gene which method comprises contacting a test sample of nucleic acid from an individual with a multiplex of diagnostic primers comprising (i) 5T variant primer 5'(N)nAAAGAC3', (ii) 7T variant primer 5'(N*)n*(N)nAAAAGC3' and (iii) 9T variant primer 5'(N*)n*(N)nAAAATC3', wherein N represents additional nucleotides which base pair with the corresponding genomic sequence in the respective allele and n is an integer between 10 and 30 and N* represents additional non-homologous nucleotides which do not base pair with the corresponding genomic sequence in the respective allele and n* is an integer between 5 and 60, in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that a diagnostic primer is extended only when the corresponding allelic variant is present in the sample; and detecting the presence or absence of the allelic variant by reference to the presence or absence of a diagnostic primer extension product.
2. A method as claimed in claim 1 and which comprises the detection of further human CFTR gene alleles by, in a separate ARMS reaction, the use of one or more of:
- DF508 mutant primer 5'(N)nACCATT3',
3849+10kb C>T mutant primer 5'(N)nTACGCA3',
N1303K mutant primer 5'(N)nTCCATC3',
1717-1G>A mutant primer 5'(N)nTAATTA3',
W1282X mutant primer 5'(N)nCAGTCA3', and
G542X mutant primer 5'(N)nTTCTCT3' wherein N and n are as previously defined, in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that a diagnostic primer is extended only when the corresponding allelic variant is present in the sample; and detecting the presence or absence of the allelic variant by reference to the presence or absence of a diagnostic primer extension product.
3. A method as claimed in claim 1 and which comprises the detection of further human CFTR gene alleles by, in a separate ARMS reaction, the use of one or more of:
- DF508 non-mutant primer 5'(N)nACCACA3',

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W1282X mutant primer 5'(N)nCAGTCA3',
1717-1 mutant primer 5'(N)nTAATTA3',
G542X mutant primer 5'(N)nTTCTCT3',
N1303K mutant primer 5'(N)nTCCATC3',
5 DF508 non-mutant primer 5'(N)nACCACA3',
DF508 mutant primer 5'(N)nACCATT3' and
3849+10kb C>T mutant primer 5'(N)nTACGCA3' wherein N and n are as previously
defined, in the presence of appropriate nucleotide triphosphates and an agent for
polymerisation, such that a diagnostic primer is extended only when the corresponding allelic
10 variant is present in the sample; and detecting the presence or absence of the allelic variant by
reference to the presence or absence of a diagnostic primer extension product.

4. A method as claimed in claim 1 and which comprises the detection of further human
CFTR gene alleles by, in a separate ARMS reaction, the use of one or more of:

15 A455E mutant primer 5'(N)nGTTGTA3',
2183AA>G mutant primer 5'(N)nGATAGC3',
3659delC mutant primer 5'(N)nCCTAGA3',
DI507 mutant primer 5'(N)nATAACT3',
1078delT mutant primer 5'(N)nTTCCTG3',
20 R347P mutant primer 5'(N)nTCTACC3',
S1251N mutant primer 5'(N)nGAAGCA3' and
E60X mutant primer 5'(N)nCAGTTA3'

wherein N and n are as previously defined, in the presence of appropriate nucleotide
triphosphates and an agent for polymerisation, such that a diagnostic primer is extended only
25 when the corresponding allelic variant is present in the sample; and detecting the presence or
absence of the allelic variant by reference to the presence or absence of a diagnostic primer
extension product.

5. A method as claimed in claim 1 and which comprises the detection of further human
30 CFTR gene alleles by, in a separate ARMS reaction, the use of one or more of:

G85E mutant primer 5'(N)nCTACGA3',
405+1G>A mutant primer 5'(N)nTAGTGA3',

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S549R mutant primer 5'(N)_nCTGACG3',

W1089X mutant primer 5'(N)_nCAAATA3' and

5 D1152H mutant primer 5'(N)_nCACTTG3' wherein N and n are as previously defined,
in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such
that a diagnostic primer is extended only when the corresponding allelic variant is present in
the sample; and detecting the presence or absence of the allelic variant by reference to the
presence or absence of a diagnostic primer extension product.

10 6. A method as claimed in any one of the previous claims and which further comprises
the use of one or more common amplification primer(s) in the presence of appropriate
nucleotide triphosphates and an agent for polymerisation, and subjecting the mixture to PCR
amplification such that a diagnostic primer is extended only when the corresponding allelic
variant is present in the sample; and detecting the presence or absence of the allelic variant by
reference to the presence or absence of a corresponding PCR amplification product.

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7. A method as claimed in any one of claims 2-6 and wherein two or more diagnostic
primers are used as a multiplex.

20 8. A method as claimed in any one of claims 2-6 and wherein all of the diagnostic
primers are used in a single multiplex reaction.

9. A method as claimed in any one of the previous claims and which further comprises
the use of one or more control primers.

25 10. A method as claimed in claim 1 and used in conjunction with any known diagnostic
CFTR gene procedure.

11. A diagnostic primer selected from any one of
5T variant primer 5'(N)_nTGTTAAAGAC3',
30 7T variant primer 5'(N*)_n(N)_nTTAAAAAAGC3' and
9T variant primer 5'(N*)_n(N)_nAAAAAAAATC3' wherein N, n, N* and n* are as defined in
claim 1 and n is an integer between 6 and 26

12. A diagnostic primer selected from any one of
 5T variant primer 5'TAATCCCCCAAATCCCTGTAAAGAC3',
 7T variant primer 5'(N*)n*TAATCCCCCAAATCCCTGTAAAAAAGC3' and
 5 9T variant primer 5'(N*)n*TAATCCCCCAAATCCCTGTAAAAAAATC3' wherein N*
 and n* are as defined in claim 1.
13. A diagnostic primer selected from any one of
 7T variant primer
 10 5'GTTAATCATTCAGCTACTACGCACCTAATCCCCCAAATCCCTGTAAAAAAGC3'
 and 9T variant primer
 5'GACTGTACGATACTCATTTATATGAAGTCAGCTACTTACCTATAGAACGCTTGC
 TAGTTTAATTCCCCCAAATCCCTGTAAAAAAATC3'
- 15 14. A set of diagnostic primers as set out in Table 1
15. A diagnostic primer selected from any one of
 DF508 mutant primer 5'(N)nACCATT3',
 3849+10kb C>T mutant primer 5'(N)nTACGCA3',
 20 N1303K mutant primer 5'(N)nTCCATC3',
 1717-1G>A mutant primer 5'(N)nTAATTA3',
 W1282X mutant primer 5'(N)nCAGTCA3', and
 G542X mutant primer 5'(N)nTTCTCT3' wherein N and n are as defined in claim 1 and n is
 an integer between 6 and 26
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16. A diagnostic primer selected from any one of
 W1282X mutant primer 5'(N)nGCAACAGTCA3',
 1717-1G>A mutant primer 5'(N)nTTGGTAATTA3',
 G542X mutant primer 5'(N)nATAGTTCTCT3',
 30 N1303K mutant primer 5'(N)nGGGATCCATC3',
 DF508 mutant primer 5'(N)nAAACACCATC3' and
 3849+10kb C>T mutant primer 5'(N)nGTCTTACGCA3' wherein N and n are as defined in
 claim 1 and n is an integer between 6 and 26

17. A diagnostic primer selected from any one of
W1282X mutant primer 5'TCTTGGGATTCAATACTTTGCAACAGTCA3',
1717-1G>A mutant primer 5'TCTCGAATTTTCTATTTTGGTAATTA3',
5 G542X mutant primer 5'AGTTTGCAGAGAAAGACAATATAGTTCTCT3',
N1303K mutant primer 5'TGATCACTCCACTGTTTCATAGGGATCCATC3',
DF508 mutant primer 5'GTATCTATATTCATCATAGGAAACACCAT3', and
3849+10kb C>T mutant primer 5'GAACATTTTCCTTTCAGGGTGTCTTACGCA3'.
- 10 18. A set of diagnostic primers as set out in Table 2
19. A diagnostic primer selected from any one of
DF508 non-mutant primer 5'(N)nACCACA3',
W1282X mutant primer 5'(N)nCAGTCA3',
15 1717-1 mutant primer 5'(N)nTAATTA3',
G542X mutant primer 5'(N)nTTCTCT3',
N1303K mutant primer 5'(N)nTCCATC3',
DF508 non-mutant primer 5'(N)nACCACA3',
DF508 mutant primer 5'(N)nACCATT3' and
20 3849+10kb C>T mutant primer 5'(N)nTACGCA3' wherein N and n are as defined in claim 1
and n is an integer between 6 and 26
20. A diagnostic primer selected from any one of
DF508 non-mutant primer 5'(N)nAAACACCACA3',
25 R117H mutant primer 5'(N)nGCGATAGACT3',
621+1G>T mutant primer 5'(N)nGAAGTATTGA3',
R334W mutant primer 5'(N)nATCATCCTGT3',
R1162X mutant primer 5'(N)nTCTGTGAGTT3',
R553X mutant primer 5'(N)nTTCTTGCTGA3' and
30 G551D mutant primer 5'(N)nGCTCGTTGTT3' wherein N and n are as defined in claim 1
and n is an integer between 6 and 26

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21. A diagnostic primer selected from any one of
R117H mutant primer 5'AGCCTATGCCTAGATAAAATCGCGATAGACT3',
621+1G>T mutant primer 5'TGCCATGGGGCCTGTGCAAGGAAGTATTGA3',
R334W mutant primer 5'CCTATGCACTAATCAAAGGAATCATCCTGT3',
5 R1162X mutant primer 5'TATTTTATTTTCAGATGCGATCTGTGAGTT3',
R553X mutant primer 5'TTATTCACCTTGCTAAAGAAATTCTTGCTGA3',
G551D mutant primer 5'GCTAAAGAAATTCTTGCTCGTTGTT3'.
22. A set of diagnostic primers as set out in Table 3
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23. A diagnostic primer selected from any one of
A455E mutant primer 5'(N)nGTTGTA3',
2183AA>G mutant primer 5'(N)nGATAGC3',
3659delC mutant primer 5'(N)nCCTAGA3',
15 DI507 mutant primer 5'(N)nATAACT3',
1078delT mutant primer 5'(N)nTTCCTG3',
R347P mutant primer 5'(N)nTCTACC3',
S1251N mutant primer 5'(N)nGAAGCA3' and
E60X mutant primer 5'(N)nCAGTTA3' wherein N and n are as defined in claim 1 and n is
20 an integer between 6 and 26
24. A diagnostic primer selected from any one of
A455E mutant primer 5'(N)nAGTTGTTGTA3',
1078delT mutant primer 5'(N)nAGGGTTCCTG3',
25 R347P mutant primer 5'(N)nTTGTTCTACC3',
DI507 mutant primer 5'(N)nGAAAATAACT3',
3659delC mutant primer 5'(N)nTAAACCTAGA3',
2183AA>G mutant primer 5'(N)nAAAAGATAGC3',
S1251N mutant primer 5'(N)nCAGGGAAGCA3' and
30 E60X mutant primer 5'(N)nAAGCCAGTTA3' wherein N and n are as defined in claim 1 and
n is an integer between 6 and 26

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25. A diagnostic primer selected from any one of
 A455E mutant primer 5'TTCAAGATAGAAAGAGGACAGTTGTTGTA3',
 1078delT mutant primer 5'CCTTCTTCTTCTCAGGGTTCCTG3',
 R347P mutant primer 5'CACCATCTCATTCTGCATTGTTCTACC3',
 5 DI507 mutant primer 5'GCCTGGCACCATTAAAGAAAATAACT3',
 3659delC mutant primer 5'ATGCCAACAGAAGGTAAACCTAGA3',
 2183AA>G mutant primer 5'CAAACCTCTCCAGTCTGTTTAAAAGATAGC3',
 S1251N mutant primer 5'GGAAGAAGTGGATCAGGGAAGCA3' and
 E60X mutant primer 5'TTAGGATTTTCTTTGAAGCCAGTTA3'.
- 10 26. A set of diagnostic primers as set out in Table 4
27. A diagnostic primer selected from any one of
 G85E mutant primer 5'(N)nCTACGA3',
 15 405+1G>A mutant primer 5'(N)nTAGTGA3',
 S549R mutant primer 5'(N)nCTGACG3',
 W1089X mutant primer 5'(N)nCAAATA3' and
 D1152H mutant primer 5'(N)nCACTTG3' wherein N and n are as defined in claim 1 and n is
 an integer between 6 and 26
- 20 28. A diagnostic primer selected from any one of
 G85E mutant primer 5'(N)nTGTTCTACGA3',
 405+1G>A mutant primer 5'(N)nTATTTAGTGA3',
 S549R mutant primer 5'(N)nCACACTGACG3',
 25 W1089X mutant primer 5'(N)nCTGCCAAATA3',
 D1152H mutant primer 5'(N)nTATCCACTTG3' wherein N and n are as defined in claim 1
 and n is an integer between 6 and 26
29. A diagnostic primer selected from any one of
 30 G85E mutant primer
 5'TAGCCATTGATGACGGAGCGATGTTTTTCTGGAGATTATGTTCTACGA3'
 405+1G>A mutant primer

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5'GATTTATGTTCTATGGAATCTTTTATATTTAGTGA3',
S549R mutant primer 5'TGGAGAAGGTGGAATCACACTGACG3',
W1089X mutant primer 5'AAGCTCTGAATTTACATACTGCCAAATA3' and
D1152H mutant primer 5'AAAGATGATAAGACTTACCAAGCTATCCACTTG3'

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30. A set of diagnostic primers as set out in Table 5